Reference: *Biol. Bull.* **183**: 278–296. (October, 1992)

Characteristics of Carbonates of Gorgonian Axes (Coelenterata, Octocorallia)

J. C. LEWIS, T. F. BARNOWSKI, AND G. J. TELESNICKI

Department of Biological Sciences, Brock University, St. Catharines, Ontario, Canada L2S 3A1

Abstract. Axial skeletons of 13 species of gorgonians were examined by SEM, X-ray diffraction, and polarizing microscopy. Calcite, though occasionally amorphous is the major biogenic carbonate of axes. Non-biogenic mineralization may be calcitic, amorphous, or aragonitic. Axes of *Plexaurella* contain numerous, lenticular, calcitic loculi of spherulitic prismatic crystals. Mineralization in *Ellisella barbadensis* is in the form of concentric layers of perpendicularly oriented, lath-shaped crystals that extend through the annulations. Numerous longitudinally oriented collagen fibers perforate the crystals. Mineralization in *Lophogorgia cardinalis* is in the form of crescentic, shield-shaped, flat, laminated plates composed of alternating layers of calcified (sheathed) and uncalcified collagen fibers. The fibrous component in all species is oriented parallel to the longitudinal axis of axes. Fine striae on transversely fractured crystals of species of *Plexaurella* and *E. barbadensis* probably represent daily growth banding. The functional associations of mineral forms with stiffness, resistance to twist, and water movement zones are discussed.

Introduction

The gross morphology of a gorgonian colony is primarily the product of its variably branching axial skeleton (Muzik and Wainwright, 1977), which is composed primarily of a collagenous matrix called gorgonin (Barnes, 1980; Goldberg, 1976). The function of the axis as a mechanical support system is based on octocorals being passive suspension feeders that collect particulate food from flowing water. The axis must be rigid enough to hold the fragile polyps off the substratum and must be able to withstand the total water velocities for the particular habitat or zone inhabited (Muzik and Wainwright, 1977). The mechanical properties of the axis can vary (Jeyasuria and Lewis, 1987) with the sclerotization of the gorgonin (Goldberg, 1976) and calcification (Kingsley and Watabe, 1987). Usually the major component of the axial skeleton is the gorgonin, composed mainly of collagen fibers in a proteinaceous matrix (Leversee, 1969). The protein matrix is largely uncharacterized, but the collagen, though modified (Goldberg, 1973, 1976; Wainwright *et al.*, 1982), is characterized as collagen.

Gorgonin is deposited extracellularly in concentric layers around a central, hollow, chambered canal that seldom exceeds 100 μm in diameter. Goldberg's (1973) micrographs of fractured surfaces show that these layers are made of collagen fibers. The fibers appear to be lath-shaped not round as is mammalian tendon (Weise, 1988). The three-dimensional fibrous texture of gorgonin is complex, but shows a preferred axial orientation (Goldberg, 1973). The axis is thus flexible (when wet) and has a high tensile strength. The mechanical properties of collagens vary widely. Different chemical and macromolecular composition as well as the organization of collagen fibers (Goldberg, 1976), profoundly influence their mechanical properties.

Though flexibility can apparently be controlled or modulated by sclerotization of the collagen within the axial skeleton, a widely used method of stiffening axes in gorgonians is extracellular deposition of carbonates within the collagen interstitial spaces (Lowenstam, 1964; Jeyasuria and Lewis, 1987). Jeyasuria and Lewis (1987) determined the Young's modulus of the axial skeletons of thirteen species of holaxonian octocorals representing twelve genera and found that they range from 0.2 Gdynes/cm^2 to 90 Gdynes/cm^2; axial stiffness also correlated well with zone-related water movement. Relative quantities of calcareous material in the axial skeletons were strongly correlated with Young's modulus, suggesting an important role for calcareous material in modulating the mechanical

Received 16 August 1991; accepted 2 July 1992.

properties of the axial skeleton. Modulation of axial stiffness through calcification would be an effective mechanism for dealing with the different hydrodynamic forces encountered at various depths.

The two major calcium salts used in skeletal structures are calcium phosphates and calcium carbonates. Usually, phosphates occur in conjunction with collagen, and carbonates in conjunction with glycoproteins. Phosphates tend to be the major form in vertebrates and brachiopods; carbonates in most other invertebrates (Vincent, 1981) including gorgonians. Jeyasuria and Lewis (1987) compared the mole percent $MgCO_3$ in $Mg/Ca(CO_3)$ of thirteen species of octocorals. Three distinct groups were found: 14–18 mole percent $MgCO_3$, 33–42 mole percent $MgCO_3$, and 71–85 mole percent $MgCO_3$. X-ray diffraction patterns for *E. barbadensis*, from the first group, showed high magnesium calcite as the crystallographic form of the carbonate mineral. The higher percentages are chemically indicative of dolomite and magnesite respectively, but crystallographic mineral type was not determined by X-ray diffraction. The substitution of smaller ions such as magnesium, carrying the same charge as calcium into a calcium carbonate matrix, increases the density of the ionic packing. This results in both higher densities and greater hardness (Vincent, 1981).

The process of biomineralization can be biogenic and mediated by the organic matrix, in which case the precipitation process is controlled by a biological system, or it can be nonbiogenic and simply biologically induced, but not controlled. The calcified loculi evident in some species of octocorals are the result of a biogenic process (Wainwright, 1988). Crystal formation by organisms is commonly controlled by an array of extracellular proteins and polysaccharides. In skeletal mineralization, these macromolecules generally form a mold or framework in which the crystals grow. The macromolecules on the surface of this organic matrix are most directly involved in regulating crystal nucleation and growth (Addadi *et al.*, 1987). The polysaccharides are generally sulfated and carboxylated (Weiner *et al.*, 1983). The type of mineral nucleated may reflect the molecular structure of the nucleation site. Weiner *et al.* (1983) have proposed that the mineral crystal form (*i.e.*, calcite or aragonite) is dependent on the nucleation site.

In this study, the morphology of the axial skeleton was examined in representatives of ten genera of gorgonians. The 12 species chosen represent an axial mineral gradient in which the value calcium + magnesium/dry weight varies from 0.15–25% (Esford and Lewis, 1990), and the mineral form based on atomic absorption spectroscopy could be aragonite, calcite, dolomite or magnesite (Jeyasuria and Lewis, 1987; Esford and Lewis, 1990). Light microscopy, polarizing microscopy, scanning electron microscopy, and X-ray diffraction were used to perform intra- and interspecific comparisons of collagen fiber orientation, calcified loculus orientation, and mineral crystallographic form within the axis, and to describe in detail the calcified aggregates in several species.

Materials and Methods

The specimens of octocorals used for this study were obtained during field trips to the reefs of Tobago, West Indies. Most of the samples were collected from the northeastern tip of the island between 1984 and 1988. The 13 species used were chosen from ten genera so as to represent a broad array of octocorallian morphological and compositional characteristics. Whole colonies were collected intact, dipped in 10% formalin, air dried, and transported to Brock University for dry storage.

Species were identified with the aid of Bayer's (1961) guide "The Shallow Water Octocorallia of the West Indian Region". They are all in the phylum Cnidaria, class Anthozoa, subclass Octocorallia, order Gorgonacea, and suborder Holaxonia. Those in the family Plexauridae include the species *Plexaura flexuosa, Plexaurella grisea, Plexaurella fusifera, Plexaurella nutans, Eunicea tourneforti, Pseudoplexaura porosa,* and *Muricea muricata;* and in the family Gorgonidae are the species *Lophogorgia cardinalis, Gorgonia ventalina, Leptogorgia virgulata, Pseudopterogorgia acerosa,* and *Pseudopterogorgia bipinnata;* and in the family Ellisellidae is the species *Ellisella barbadensis.*

Cortex and adherent material were removed and the axis exposed. Sections were then cut from the axis, either with a jeweler's saw or a fine-toothed, circular, Drommel blade. Samples meant to show cross-sectional and longitudinal surfaces were taken from bases, mid-sections, and tips of each colony. Three series were cut. The first set was dipped in a sodium hypochlorite solution, which dissolved the organic matrix and exposed calcified inclusions. The second series was etched in 12% trichloroacetic acid, dissolving the calcified aggregates. The third series of samples were buffed with an emery board. Each species was then examined under dissecting and compound microscopes.

Measurements on those samples containing substantial calcified aggregates were made with an optical micrometer. The proportions of organic matrix to calcified structure were derived from cross sections: the amount of calcified material was compared with the amount of organic matrix along a series of radial lines.

Alizarin red S staining was also done to indicate the extent of mineralization. Samples were etched in bleach for up to 30 min, then washed in deionized water. They were then placed in a vial with an Alizarin Red S stain for 7–10 min and rinsed with deionized water. This stain colors calcite and aragonite red, and dolomite blue. The

procedure was also used on thin translucent shavings cut from unetched axes with a #10 scalpel blade. Shavings were examined with a light microscope at a magnification of 1000×.

Small subsamples, about 1.5 cm^3, were stripped of all adherent matter and immersed in sodium hypochlorite solution until all of the organic matter dissolved. The remaining particulate matter was then rinsed several times with deionized water. The mineral was then filtered and collected on #1 Whatman filter paper. With a mortar and pestle, the residual powder was ground to a fine consistency. A Picker 2087 generator was used to diffract an X-ray beam through the powder, which had been affixed to a glass plate with silicon vacuum gel. A graphical readout representing theta angles was produced. Diffraction peaks were then compared to standards so that the mineralized crystal lattice of each of the samples could be identified.

No diffraction pattern was generated from some of the samples. Powder from these samples was packed into a 0.03 mm capillary tube, and an X-ray beam was passed through the specimen using a Nonius CAD–4 automated, single crystal X-ray diffractometer. The X-ray beams are diffracted directly on to a film plate as diffraction rings. From the spacing between rings, the theta angles were calculated for comparison to known mineral standards.

Scanning electron microscopy (SEM)

Various preparative techniques were utilized to expose the outer, inner, lateral, longitudinally fractured, and the transversely fractured surfaces of the axial skeleton. Fractured transverse surfaces were obtained with a fine handsaw, or by bending or twisting the axial skeleton perpendicular to the longitudinal axis until it fractured. Longitudinal surfaces were exposed by splitting cross-sectional specimens with a scalpel blade. The fractured specimens were examined untreated or after further treatment. To remove the gorgonin and collagen fibres and to expose the calcareous matrix, specimens were treated with concentrated hypochlorite bleach (Chlorox® or Javex®) for various periods, from 30 s to 20 min, depending upon the amounts of organic matrix to be removed. To expose organic matrix and remove the calcareous components, specimens were treated with 10% HCl or 2% ascorbic acid. To remove large amounts of the calcareous material relatively rapidly or to free collagen fibres from the gorgonin, concentrated HCl was used for intervals ranging from a few minutes to several hours. When only a relatively light etching of a fractured or exposed calcareous surface was desired, dilute 2% ascorbic acid was used for short periods, 30–240 s. Combinations of these treatments were also used to determine relationships between calcareous and organic matrices. Powder residue was also examined.

Specimens were mounted on aluminum SEM stubs with double-sided mounting tape, gold sputter-coated (Polaron PS-3) for 60–120 s, and examined in a Hitachi S-570 scanning electron microscope.

Results

The gorgonian axes examined were morphologically diverse. They ranged from axes that were white and heavily calcified, through those with white, crystalline aggregates embedded in brown gorgonin, to those with no apparent mineralization and with gorgonin that varied from brown and fibrous to almost glassy black. Dried axes exhibit some artifacts due to dehydration. There is often some longitudinal checking or cracking of the gorgonin, but it is usually not severe. Heavily mineralized axes exhibit the least artifactual splitting. Axes with what appears to be amorphous, dense gorgonin show moderate checking. Axes that are readily distinguishable as fibrous usually show the greatest degree of artifactual splitting. Measurements taken with calipers revealed no significant differences in axis diameter between fresh, dehydrated, and rehydrated samples. The 12 species examined can be readily separated into 3 groups on the basis of mineralization.

Species with high Ca + Mg values (*i.e.*, 10–25% Ca + Mg/dry wt; Jeyasuria and Lewis, 1987; Esford and Lewis, 1990) contain easily distinguishable calcified aggregates. The species making up this group include *Ellisella barbadensis, Plexaurella nutans, Plexaurella grisea,* and *Plexaurella fusifera.* With the exception of *E. barbadensis,* the mineralized loculi in cross-sectional aspect are semi-lunate and oriented so their concave surfaces face the central core of the axis. In longitudinal aspect, loculi are lenticular in shape. The number of loculi per unit area is greatest near the central region of the axis. Individuals of *E. barbadensis* have a mineralized central rod surrounded by mineralized cylinders reminiscent of a single bone osteon. As with the species of *Plexaurella,* the proportion of calcified material to organic matrix decreases distal to the axis center. The cylinders near the axial periphery deform into sickle-shaped aggregates.

The percentage of cross-sectional area occupied by mineralized aggregates varies with species and longitudinal position along the axis. The approximate percentages of mineral aggregate are: *E. barbadensis*—tip 94%, mid-colony 81%, base 46%; *P. grisea*—tip 89%, mid-colony 63%, base 29%; and *P. nutans*—tip 85%, mid-colony 57%, base 32%; and *P. fusifera*—tip 75%, mid-colony 53%, base 37%. In all cases, the proportion of gorgonin to mineral increases toward the base.

Those species with a moderate amount of Ca + Mg (*i.e.*, 2–10% Ca + Mg/dry wt) (Jeyasuria and Lewis, 1987; and Esford and Lewis, 1990) include *Leptogorgia virgulata, Lophogorgia cardinalis, Pseudopterogorgia bipinnata,* and *Pseudopterogorgia acerosa.* These species

contain bundles of relatively large collagen fibers. Mineral is associated with the fiber bundles of *Pseudopterogorgia bipinnata* and *Pseudopterogorgia acerosa* (Fig. 1a). Whether the collagen fibers themselves are mineralized, or are encased by mineral, is not clear. Bleach etching reveals thin, cylindrical, calcified sheets between the layers of organic matrix of both *Leptogorgia virgulata* and *Lophogorgia cardinalis*.

In contrast, those octocorals with Ca + Mg contents of 1–<0.1% Ca + Mg/dry wt (Jeyasuria and Lewis, 1987; Esford and Lewis, 1990) appear in cross-section to be composed of fibrous gorgonin. No mineral aggregates are apparent. Included in this group are *Plexaura flexuosa*, *Muricea muricata*, and *Eunicea tourneforti*. Though *Gorgonia ventalina* has a Ca + Mg content within the moderate range (2–10%) of the previous group, it is morphologically similar to this group. Despite apparent lack of mineralization, when all organic material is removed by hypochlorite digestion, a fine, crystalline residue remains. Most of these are similar to crystals noted in the hollow core of axes on SEM examination. Crystals separated from the matrix are highly variable in shape (Fig. 1b, c, d). Individuals of *M. muricata* exhibit crystals roughly scalenohedron in shape, an alternate habit or form of calcite (Fig. 1b). In *Plexaura flexuosa* (Fig. 1c) and many other species, crystals with sharp edges and regular geodesic forms characteristic of both nonbiogenic mineral deposition and calcite can be found. Some of the crystals in *Eunicea tourneforti* (Fig. 1d) are needle-like and characteristic of aragonite.

In every species, the points of light extinction as determined from polarizing microscopy with crossed Nicholls' filters, indicate a fibril orientation parallel to the long axes of the colony. The extent of extinction was high, and the angles of light extinction occurred at consistent angles of horizontal stage rotation at 0° and 90°. In crossed Nicholls' polarizing microscopy, polarizing filters are positioned above and below the specimen and are oriented at 90° to each other. Parallel-oriented polarizing elements in the specimen will cause light extinction when aligned with the polarizing planes of either filter. These trends were equally apparent in all species, and the interlocular organic fibrillae of the heavily calcified samples also show a distinct uniform orientation.

The X-ray diffraction patterns indicate high magnesium calcite as the crystallographic mineral form in *Plexaurella fusifera*, *Plexaurella nutans*, *Plexaurella grisea*, *Ellisella barbadensis*, *Pseudopterogorgia acerosa*, *Pseudopterogorgia bipinnata*, and *Muricea muricata*. Amorphous mineral, that which does not produce a clear diffraction pattern, was found in *Lophogorgia cardinalis*, *Leptogorgia virgulata*, *Plexaura flexuosa*, and *Gorgonia ventalina*. The mineral from *Eunicea tourneforti* was found to be aragonitic (Table 1).

Aliziran red staining of heavily calcified species, such as *Plexaurella fusifera*, *Plexaurella nutans*, *Plexaurella grisea*, and *Ellisella barbadensis*, showed good differentiation. The high magnesium calcite loculi stained red, and the interstitial matrix remained unstained (Table 1). The moderately calcified species, such as *Leptogorgia virgulata*, *Lophogorgia cardinalis*, *Pseudopterogorgia bipinnata*, *Pseudopterogorgia acerosa*, and *Gorgonia ventalina* contain calcified, lath-like sheets rather than discrete loculi. They stained with less differentiation. It appears that the mineral is contained within or around the collagenous fibers, producing the impression of widespread mineralization in low concentration. Axes of *Plexaura flexuosa*, *Muricea muricata*, and *Eunicea tourneforti* did not stain with Aliziran red, an indication that no mineralization occurs in these species. Powder, collected as a residue after the organic matrix was digested, yielded crystals from each species. All stained positively (red); but species such as *M. muricata* and *Eunicea tourneforti* produced limited amounts of crystal, little of which stained.

Detailed scanning electron microscopic examination was done on the calcareous components of axes of several heavily mineralized species.

Plexaurella nutans, P. grisea *and* P. fusifera

Because the carbonate deposits in the axes of these three species showed only minor differences, they are treated as a single group.

Axial skeletons of species of *Plexaurella* are composed of fibrous gorgonin and calcareous loculi. In a bleach-etched cross section of an axis of *Plexaurella nutans*, the large number of loculi and their tight packing are clearly evident, and they form a substantial component of the axis (Fig. 2a). Elongate loculi are semi-lunate, through anvil, to kidney-shaped in cross section (Fig. 2b). Widths vary from about 40 μm to more than 250 μm. Inner (toward the hollow core), outer and lateral surfaces of loculi are morphologically distinct (Figs. 2b, c). Surfaces of transversely fractured loculi exhibit fan-shaped, crystal patterns that radiate peripherally (Figs. 2c, d). At high magnification, after light ascorbic-acid etching, fine lateral striations that vary in width from 0.3–0.5 μm are visible in all three species (Fig. 2d).

Individual loculi are elongate, pallisade-shaped, longitudinally oriented with respect to the axis, and occasionally branch (Fig. 3a). The outer surface of a loculus is composed of the ends of the crystal plates (Fig. 3b), horizontally oriented and irregularly fusiform. Exposed ends of crystals appear as small, thin, narrow transverse plates that terminate at the outer surface of the loculus (Fig. 3b). Localized groups of plates exhibit similar orientations slightly different from adjacent groups (Fig. 3b). The inner surfaces of loculi exhibit longitudinally oriented

Figure 1. (a) Surface of a fibroid crystal aggregate from the matrix of the axis of *Pseudopterogorgia bipinnata*. The structures bear the impressions of collagen fiber layers between which they occur. X-ray diffraction pattern indicates calcite. 2000×. (b) Mineral aggregate from the hollow axial core of *Muricea muricata*. X-ray diffraction pattern indicates calcite. Crystal forms are scalenohedral, an alternate calcite form characteristic of non-biogenic deposition. 1300×. (c) Calcareous crystals from the hollow axial core of *Plexaura flexuosa* of geodesic form indicative of typical, non-biogenic, calcite deposition. X-ray diffraction pattern is amorphous. 3800×. (d) Mineral aggregates from the hollow core of *Eunicea tourneforti*. The needle-like crystal form is characteristic of non-biogenic aragonite deposition. X-ray diffraction pattern indicates aragonite. 1200×.

Table I

Staining properties, compositional, and morphological characteristics of investigated species of octocorallians relative to water movement zones

Species	Aliziran red stain	Carbonate crystal type	Gross mineral form	Axis matrix	Water movement zone	Colony size and branch form
Ellisella barbadensis	+	Calcite	heavily calcified cylinders	fibrous	current, deep	large, whip
Plexaurella nutans	+	Calcite	lenticular loculi semi-	fibrous,	surge,	large, long thick branches
Plexaurella grisea	+	Calcite	lunate in cross section	homogeous	moderate	large, long moderate branches
Plexaurella fusifera	+	Calcite				medium, moderate branches
Leptogorgia virgulata	+	Amorphous	thin crescentic platelets	fibers in layers	surge,	small, elongate thin branches
Lophogorgia cardinalis	+	Amorphous	between fiber layers		deep current, deep	small, planar, highly branched
Pseudopterogorgia acerosa	+	Calcite	small aggregates interspersed with fibers	bundles of fibers in regular	surge, moderate	large, plumose, feathery
Pseudopterogorgia bipinnata	+	Calcite	and in hollow core	layers	surge, deep	large, bipinnately plumose
Gorgonia ventalina	+	Amorphous	small aggregates	sheet-like cylinders	breaker, shallow	large, fan, reticulum
Muricea muricata	−	Calcite	no mineral in matrix,	heavily fibrous	surge,	moderate, bottle brush
Plexaura flexuosa	−	Amorphous	crystals in hollow core	cylinders	moderate	moderate, elongate branches
Eunicea tourneforti	−	Aragonite			to shallow	moderate, candelabra, thick branches

grooves relative to the axis (Fig. 3c). These vertical striae are sometimes punctuated with fine granular areas. In all species, inner locular and lateral surfaces show similar longitudinal grooves, their widths ranging from 0.4–0.8 μm. Layers of gorgonin surround the individual loculi and are 8–50 μm thick. Longitudinally oriented collagen fibers approximately 0.4–8.0 μm in diameter are similar in diameter to the longitudinal grooves found on the inner locular surfaces in all 3 species (Fig. 3d). A longitudinally fractured loculus of *Plexaurella* reveals radiating crystal plates that extend from the inner surface to the outer edge of the loculus (Fig. 3e). Secondary branching of the individual crystal plates occurs. The apices of the crystal aggregates occur on or in the inner surface layer as distinct spherulitic units with the crystal plates radiating outward from the inner surface layer.

A classification of molluscan shell microstructure was created by integration of the nomenclature for brachiopods with that of mollusks (Carter and Clark, 1985). This was done to eliminate the considerable overlap and inconsistencies in the application of microstructural terminology found even within single molluscan classes. The classification system integrated by Carter and Clark (1985) will be used to describe the microstructure of Gorgonian axial skeleton calcification because of its relevance to invertebrates, though other authors (Vincent, 1982; Simkiss and Wilbur, 1989) have used other terminologies in their descriptions of similar microstructural types found in vertebrates.

Ellisella barbadensis

When bleach-etched, the cross-sectional surface of the axis shows extensive calcareous material and distinct concentric annulations, about 20–60 μm wide (Fig. 4a). The acid-etched cross-sectional surface (Fig. 4b) from which the calcareous material has been removed shows the high density of collagen fibers that compose a major portion of the axial skeleton. The collagen fibers freed from the calcareous material mat together and form protruding ridges (Fig. 4b). Large numbers of longitudinally oriented collagen fibers penetrate the calcareous material of the annulations (Fig. 4c). Annulations are composed of individual, tightly packed crystals or aggregates oriented perpendicular to the longitudinal axis of the colony (Fig. 4d). Crystals or crystal aggregates are elongate, lath-shaped, and extend entirely across an annulation. In cross section or end-on view, they are usually square and approximately 3×3 μm (Fig. 4e).

Numerous longitudinally oriented collagen fibers (Fig. 5a) are 0.2–0.4 μm in diameter. They perforate the carbonate aggregates (Fig. 5b, c). Figure 5b shows the ends of the collagen fibers protruding through the carbonate structure. Figure 5c shows the perforations remaining in the carbonate subsequent to collagen removal by bleach. A light ascorbic acid etch subsequent to transverse fracture and bleach etching reveals a series of fine, transverse striae 0.2–0.4 μm wide on the crystalline surface (Fig. 5c).

Outer and inner vertical surfaces of annulations display a multilaminar layer of fine-grained, longitudinally

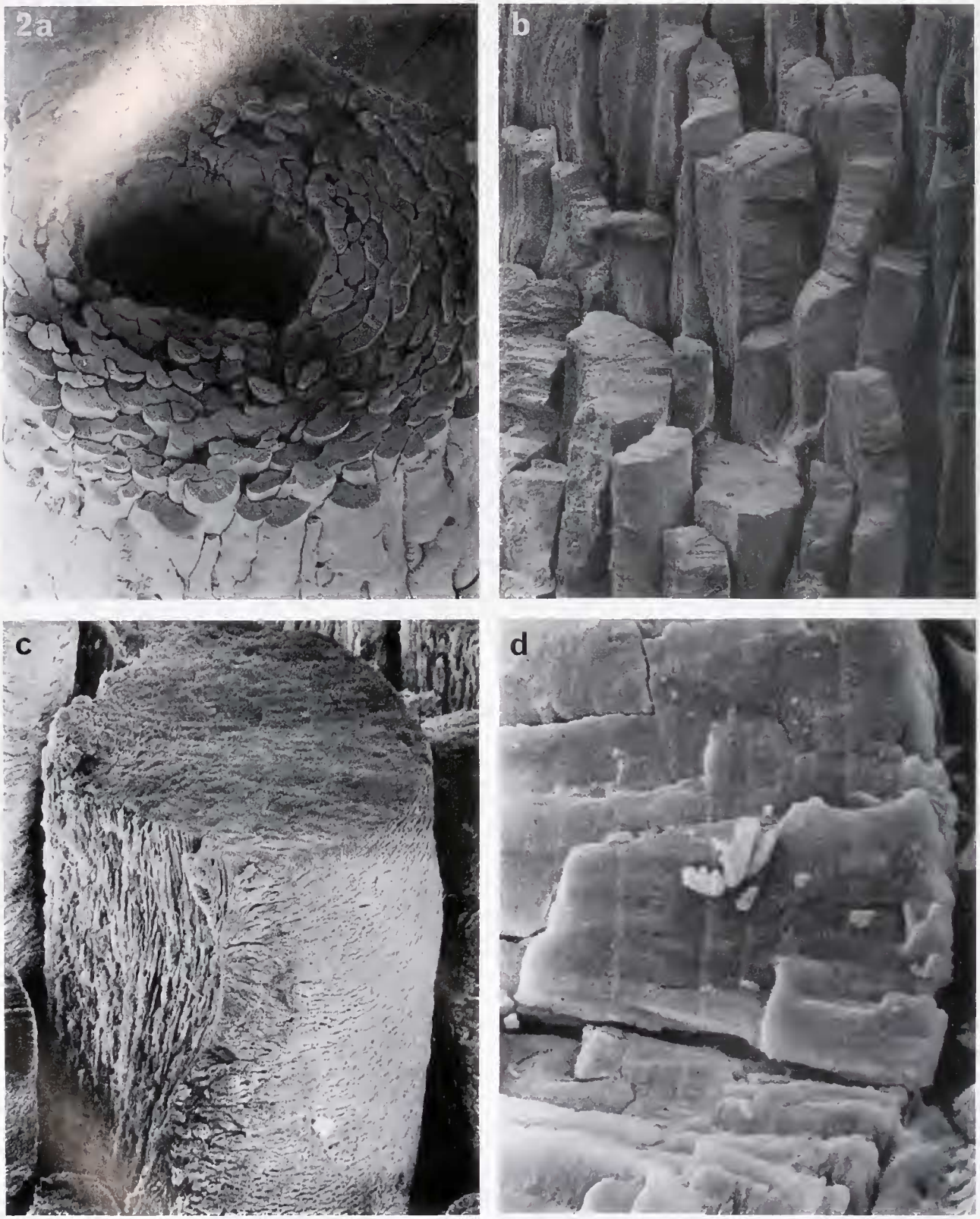

Figure 2. (a) Cross section of a bleach-etched (gorgonin removed) axis of *Plexaurella nutans*. Note the hollow core and numerous, tightly packed, semi-lunate calcareous loculi. 25×. (b) Bleach-etched (gorgonin removed), transversely fractured loculi of *Plexaurella nutans*. Inner, outer, and lateral locular surfaces are apparent. Note radiating, fan-like pattern on both transversely and longitudinally fractured surfaces. 160×. (c) Bleach-etched single loculus of *Plexaurella grisea*. Note the fibroid pattern on the inner locular surface, the stacked, fusiform, crystal plate ends of the outer locular surface, and the radiating fan pattern on the transversely cleaved surface. 950×. (d) Lightly ascorbic-acid etched, transversely cleaved locular surface of *Plexaurella fusifera*. Note the fine, vertical striae with a periodicity of about 0.3 μm that may represent episodic carbonate deposition. 4700×.

oriented crystals (Fig. 5d). There are usually a minimum of two sublayers on each surface of an annulation.

Lophogorgia cardinalis

Numerous, crescentic, lamellar, calcareous plate loculi are a major component of the axial skeleton (Fig. 6a). Removal of gorgonin and collagen by bleach etching eliminates the overlying support for the calcareous components of the loculi. Subsequent dehydration, necessary for examination in the SEM, introduces artifactual fractures in the plates and breaks them into smaller, irregular blocks (Fig. 6b). The shield-like loculi are irregular in outline and have connections with neighboring locular plates (Fig. 6c). The outer surface of locular plates is composed of fine-grained, generally longitudinally oriented crystals or aggregates (Fig. 6d). The interlocular gorgonin contains longitudinally oriented, layered collagen fibers (Fig. 6e) 0.1–0.3 μm in diameter.

Viewed edge-on, the longitudinally fractured plates are seen to branch laterally in a pattern reminiscent of cardiac muscle fibers and to form fused connections with adjacent plate loculi (Fig. 7a). The loculi are also laminated (Fig. 7a, b) with alternating layers of smooth, fine, longitudinally oriented crystals, or aggregates, and gorgonin-collagen (Fig. 7b). A transverse fracture of a loculus clearly reveals the lamination (Fig. 7c). The centers of many crystals are hollow (Fig. 7c). Acid etching, to remove the carbonate, leaves the fused and melted ends of the dense gorgonin-collagen layers of the lamellar plate as readily apparent layers in Fig. 7d.

Discussion

Octocoral axes are composed of a limited number of structural elements. Principally they contain variable amounts of flexible collagen fibers, embedded in a pliant, proteinaceous matrix, and minerals that exist in a variety of crystal forms and aggregate shapes (Kingsley and Watabe, 1984). These components are assembled in a surprisingly large range of forms (Telesnicki, 1990; Barnowski, 1991), the functional significance of which is dimly known at present. Functional relationships can be better understood if structure is known and can be used to complement, explain, and infer functional interaction. Detailed SEM examination of the carbonate inclusions in three genera has revealed three substantively different mineralization types from which some functional inferences may be derived.

Genus Ellisella

The axis of individuals of *Ellisella barbadensis* is extensively mineralized (about 30%/dry weight) by high magnesium calcite (Jeyasuria and Lewis, 1987; Esford and Lewis, 1990). Only when it is decalcified does the large collagen fiber content become evident. The axis consists of numerous, smooth concentric annular layers reminiscent of a single osteon from bone. The principal individual crystalline components of the annulations are elongate, lath-shaped, perpendicularly oriented with respect to the axis, and they extend across the entire width of the annulus. Whether these are single crystals or multiple crystal composites was not determined. These units, stacked like two-by-fours in a lumber yard, are highly resistant to compressional forces, but like bricks in a wall held only by mortar, provide little resistance to tensional forces which would tend to lift them apart. Tensional forces are accommodated by the numerous, high tensile strength, uncalcified, collagen fibers (Moss, 1964) that perforate the laths. These longitudinally oriented fibers are surrounded by calcareous material and appear tightly incorporated into it. Individual collagen fibers extend through a minimum of several laths, if not the entire length of the annulus. They can be thought of as analogous to the effectively non-extendible, iron reinforcing rods in concrete that strengthen it in tension. This construction produces the stiffest, shallow-water gorgonian axis (Jeyasuria and Lewis, 1987; Esford and Lewis, 1990) that has been recorded to date. It also has a comparatively high torsion modulus (Jeyasuria and Lewis, 1987) despite the flexibility of the collagen fibers. That is probably a result of the manner in which the lath-like, crystal aggregates are stacked, but this was not examined extensively.

The elongate, whip-like individuals of *Ellisella barbadensis* generally occur well below wave base in areas of current-generated water movement only. Forces generated by such currents are high (Roberts *et al.*, 1975), and this is one reason for the high stiffness of the axis. The other is as follows. The majority of shallow water gorgonians occur in the surge zone where wave action returns them through the upright position approximately every 12 s (open ocean wave period). Below wave base, where *Ellisella barbadensis* occurs, currents can be anywhere from constant and unidirectional through intermittent and multidirectional. In most tidal locations, bidirectional currents alternating about twice per day could be expected. In any case, the current is comparatively constant for relatively long periods (a few h). A comparatively stiff axial skeleton is required to maintain the polyps in a feeding position in the water column, and to keep the colony off the substratum under these conditions.

Fine striae, approximately 0.3 μm in width, evident on cross sections lightly etched with ascorbic acid probably represent episodic carbonate accretion. No data have been published on the rate of elongation of *Ellisella barbadensis*, let alone on the rate of increase in its diameter. The striae could represent daily accretion rates; in which case, the yearly increase in diameter would be about 0.25 mm. If they represented growth associated with different tidal

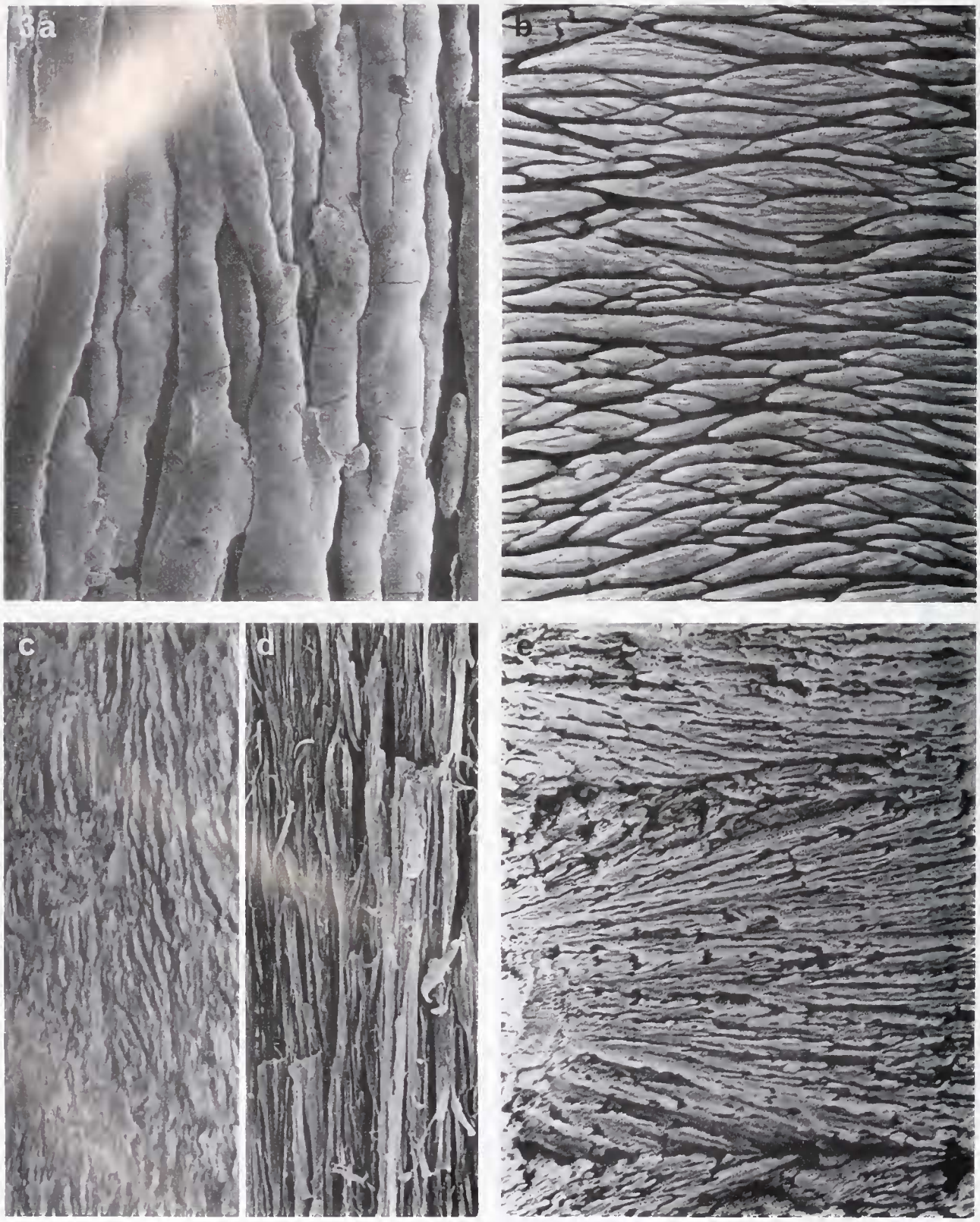

Figure 3. (a) Bleach-etched outer loculi of *Plexaurella grisea* are elongate, pallisade-shaped, longitudinally oriented, occasionally branch, and fuse with other loculi. Note in lower left corner the spherical, crystalline units that have not yet fused completely into a mature loculus. 100×. (b) Bleach-etched outer locular surface of *Plexaurella fusifera* composed of outer ends of the crystal plates. Most are fusiform. 4700×. (c) Bleach-

current regimes, of which there would be about four per day, the increase in diameter would be approximately 1 mm/year. It could be that only currents from one direction carry enough nutrient to permit carbonate deposition, or that nocturnal currents do so. In those cases there would be two striae per day deposited and an accretion rate of 0.5 mm/year. These are ahermatypic organisms (Goldberg, 1973), therefore they are dependent on "captured" prey for nutrients. Other scenarios could also account for the striae. If the diameter, including that at the base, accretes at a constant rate that does not change with age, then an individual with a basal thickness of 1 cm (not uncommon) could be somewhere between 10 and 40 years of age. This is, of course, a very crude estimate.

A series of micrographs of randomly taken specimens cannot be used to prove that dynamic processes occur in a particular sequence. Nevertheless, many micrographs of annuli in various stages of development have been examined, and we are now confident that annulus formation can be outlined. The formation of an annular layer begins with the deposition of fine, "seed crystals" over the smooth outer surface of a previous, inner annulation. The fine crystals initially have a random, granular appearance. As collagen fibers are synthesized and encapsulated within the fine seed crystals, the layer becomes longitudinally striated with impressions of the collagen fibers. It is on the outer surface of this "seed-crystal" layer that the thin perpendicularly oriented annulation crystals nucleate. Small, 'initial', annular crystals nucleate on the surfaces of the fine crystals. As the fine annular crystals grow outward, they become oriented perpendicularly by lateral interference from adjacent crystals. These fine crystals merge to form the larger, lath-shaped, perpendicularly oriented crystal aggregates that span the annulus. Collagen fiber synthesis and deposition must occur prior to crystal growth, since the longitudinally oriented collagen fibers are incorporated within the crystalline material. When annular crystal growth ceases, the skeletogenic cells of the axial epithelium secrete another layer of fine, capping crystals.

Genus Plexaurella

Axes of *Plexaurella nutans, P. grisea* and *P. fusifera* are extensively mineralized (about 20%/dry weight) by calcite of the high magnesium variety (Jeyasuria and Lewis, 1987; Esford and Lewis, 1990). In these species, crystalline aggregates (loculi), which are lenticular and fusiform, can be up to 5 mm in length and are semi-lunate in cross section. They are embedded longitudinally in the gorgonin. As in all the gorgonians examined, the collagen fibers course longitudinally through the proteinaceous matrix of the gorgonin, but do not penetrate the crystalline aggregates. The loculi are solid, crystalline structures composed of regular, spherulitic, prismatic units similar to those of molluscan shell (Carter and Clark, 1985).

Species of *Plexaurella* occur in the surge zone where they are subjected to both wave and current-generated water movements that produce high forces in storm conditions. Normally, forces generated in the surge zone are lower than those encountered either in deeper water (produced by current only), or in the shallower breaker zone (Roberts *et al.*, 1975). In addition to the back and forth sway from wave action, there is also a twisting motion that is amplified by current. Species of *Plexaurella* are generally large (some up to 2 m in height) with comparatively long, thick branches. The moderately high stiffness and moderate torsion resistance of these species accommodate them to the forces in the surge zone. Separation of the collagenous and mineral phases, with the retention of approximately similar proportions of carbonate (Jeyasuria and Lewis, 1987; Esford and Lewis, 1990), may lower the stiffness from that of *Ellisella barbadensis*. The lenticular crystalline loculi probably also stiffen the axis in the manner of filler particles (Koehl, 1982) and maintain stiffness in a moderately high (for gorgonians) range. The semi-lunate, cross-sectional shape of the loculi probably allows species of *Plexaurella* to rotate somewhat in response to twisting forces and results in a moderately low torsion modulus (Jeyasuria and Lewis, 1987).

Fine striae, about 0.4 μm in width, evident on lightly ascorbic acid-etched cross sections of loculi, again probably represent episodic carbonate accretion. No data have been published on growth rates of species of *Plexaurella*, but they appear to be very slow growing (circa 1 cm/year; Paul Yoshioka, pers. comm.). If these striae are comparable to those from *Ellisella barbadensis*, and assuming that one stria represents accretion over the same time period in both species (which may well not be the case),

---

etched inner locular surface of *Plexaurella nutans* with longitudinal grooves between fine crystals. Irregular, rosette-like patterns are thought to represent nucleation sites for spherulitic crystal aggregates of the loculus. 2300×. (d) Surface of the longitudinally-fractured gorgonin layer between loculi of *Plexaurella nutans* with longitudinally oriented collagen fibres. Note that the diameters of the collagen fibers correspond to the groove diameters in 3c. 2300×. (e) Lightly ascorbic-acid etched, longitudinally fractured loculus of *Plexaurella nutans*. Several spherulitic crystal units are evident with crystal plates radiating fan-like from an inner nucleation site out to the outer edge of the loculus. Note that some crystal plates branch and the series of cavities where adjacent plates interfere with plate growth. X-ray diffraction pattern indicates crystalline calcite. 3750×.

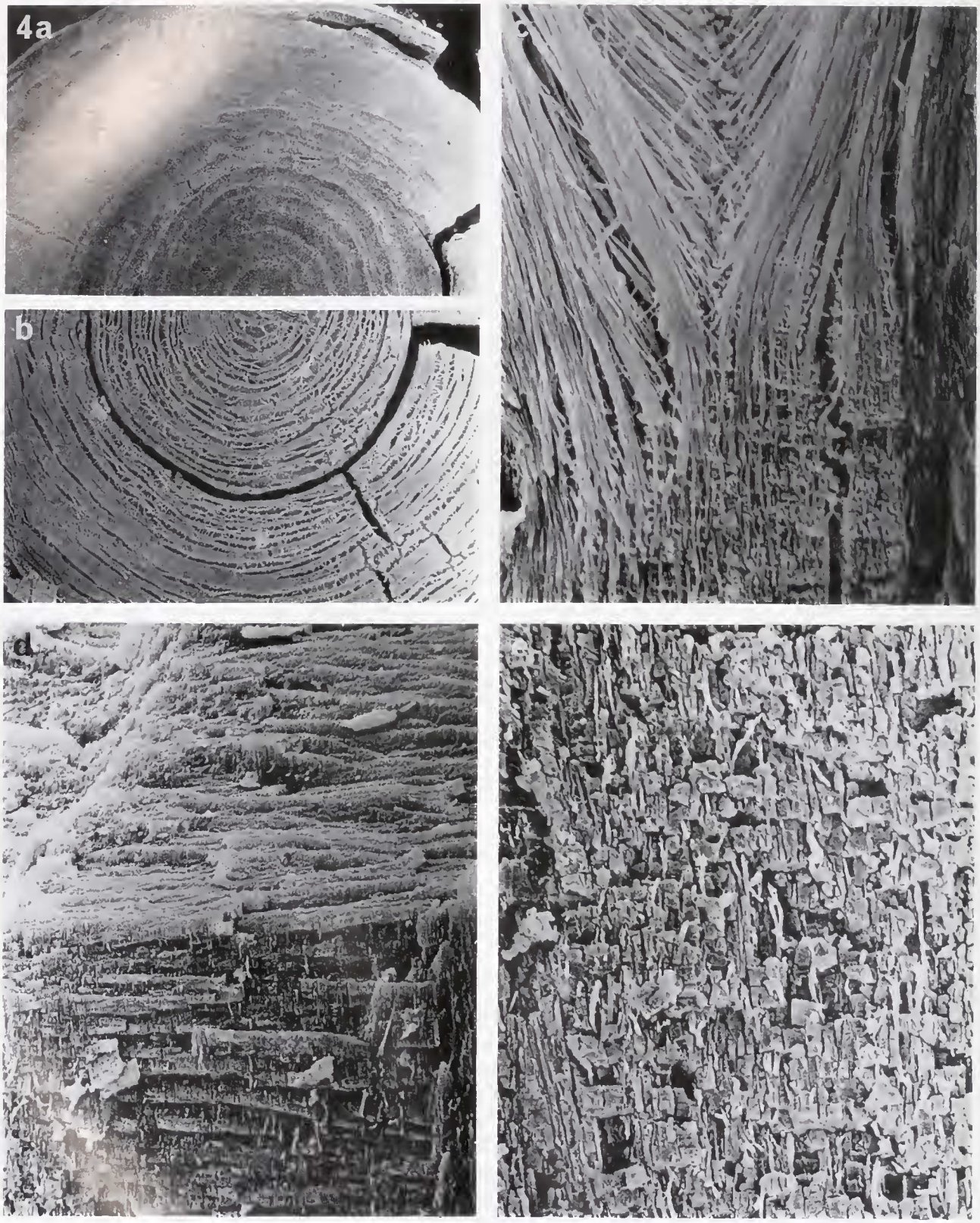

Figure 4. (a) Bleach-etched (gorgonin removed) cross section of the axis of *Ellisella barbadensis*. Note the heavily calcified annular rings of uneven diameters. 25×. (b) Acid-etched (calcareous matter removed) cross section of the axis of *Ellisella barbadensis*. Note the concentric ridges formed by extensive collagen fibers matted together. 25×. (c) Acid-etched, longitudinally fractured annular ring showing longitudinally

then the growth rate of the species of *Plexaurella* is about 25% faster. Species of *Plexaurella* are hermatypic (Ramsaroop, 1977) and contain symbiotic zooxanthellae principally in the tissues of their polyps. Hermatypic forms may gain additional nutrition and therefore increased growth potential from this symbiotic relationship between them (Pearse and Muscatine, 1971). Though the exact mechanisms of crystal growth, induction and cessation are not known, growth has been related to diurnal cycles. Chalker and Taylor (1978) and Gladfelter (1984) reported that calcification in scleractinian corals is directly proportional to photosynthesis. Daily variations in skeletogenesis are due, not only to daily changes in solar irradiance, but also to daily changes in the efficiency of the symbiotic algal photosynthesis. Zooxanthellae of *Gymnodinium microadriaticum* exhibit an endogenous circadian rhythm (Chalker and Taylor, 1978). Reduced organic carbons produced by algal photosynthesis are used as a substrate for the formation of an organic matrix upon which crystal growth occurs (Kingsley and Watabe, 1984). Narrow lines produced by capping proteins that halt crystal growth in response to a variety of factors in scleractinians are similar to those noted in this species (Chalker and Taylor, 1978).

Again, knowing the pitfalls, we feel that enough loculi in various stages of formation have been examined to permit an outline of loculus formation. It probably begins with deposition of fine, granular crystals over and around loose collagen fibers. The collagen fibers do not appear to be calcified, but are surrounded by the fine-grained crystals. This forms a thin, seed crystal layer. Deposition of the fine seed crystal layer could be induced by coating the collagen fibers with glycosaminoglycans (GAGs) which are known to concentrate calcium, creating the supersaturation necessary for nucleation on the collagen fibers (Addadi *et al.*, 1987). Within the thin seed-crystal layer, spherulitic nucleation sites occur at various points. Crystal growth initially radiates in all directions, but interference is encountered in most directions. The inner surface, seed-crystal layer, and adjacent nucleation sites restrict most of the crystal plate elongation to a direction perpendicular to the initiation layer. This method of crystal plate elongation initially results in the nodular appearance of loculi in the early stages of formation, with each nodule representing crystal plate growth from a single nucleation site. As locular thickness increases through crystal plate elongation and secondary branching, the radiating crystal plate ends of the individual nodules become increasingly interdigitated and more difficult to distinguish. When the loculus is near the final stage of formation, the outer surface loses the nodular texture and forms a relatively smooth, rounded, outer surface. At this stage, the rounded outer locular surface is composed of the sharp-pointed, lath-shaped (Carter and Clark, 1985) crystal plate ends.

The transversely fractured surface has a fan-like crystal pattern which radiates outwards only from the inner surface nucleation sites. This radiating pattern is due to the outward growth of the crystal plates, the ends of which create the outer surface.

On longitudinally fractured surfaces of individual loculi the fan-like crystal plate growth is readily apparent. Again, the radiating pattern from the inner surface occurs only from discrete nucleation sites. Secondary branching of the crystal plates occurs at regular intervals along the edges of the elongating crystal plates. The vertical rows of holes evident in the ascorbic acid-etched longitudinally fractured surfaces are located where there is interference between the secondarily branching plates. These secondary interference edges appear as 3.0 μm wide bands in the transversely fractured surfaces.

Biomineralization requires four conditions: (1) fluid supersaturation, (2) crystal nucleation, (3) crystal growth, and (4) a mechanism for controlling crystal growth so it can be structurally useful to an organism (Simkiss and Wilbur, 1989). Little is known about the calcium-carbonate secreting skeletogenic cells of gorgonian axes or of their ability to control crystal deposition. Kingsley and Watabe's (1984) work on *Leptogorgia virgulata* (a related gorgonian), showed that calcium ions are transported from the external environment to the axis. Calcium ion transport out of the axis through the axial epithelium was mediated by Ca-ATPase (Kingsley and Watabe, 1984). In 1987, Kingsley and Watabe isolated carbonic anhydrase activity adjacent to calcifying structures in the axis. Thus, a mechanism for fluid supersaturation exists in gorgonians. The mineral crystals probably grow on a framework of extracellular proteins and polysaccharides (Weiner *et al.*, 1983). Addadi *et al.* (1987) demonstrated that sulphates and beta-sheet structured carboxylates from the acidic matrix macromolecules cooperate in oriented calcite crystal nucleation.

oriented collagen fibers. Thin horizontal plates are remnants of calcareous plates. 1900×. (d) Annular ring with transverse and longitudinal fracture surfaces. Crystal aggregates oriented horizontally are tightly stacked (like lumber), elongate, lath-shaped and extend entirely across the annular ring. Thin vertical fibers are broken collagen fibers. X-ray diffraction pattern indicates calcite. 950×. (e) Outer surface of a fractured annulation. Crystal aggregates are oriented in end-on view and are perpendicular to the longitudinal axis of the colony. In cross section most are square (3 × 3 μm). Vertical fibers are collagen fibers, many of which are broken. 950×.

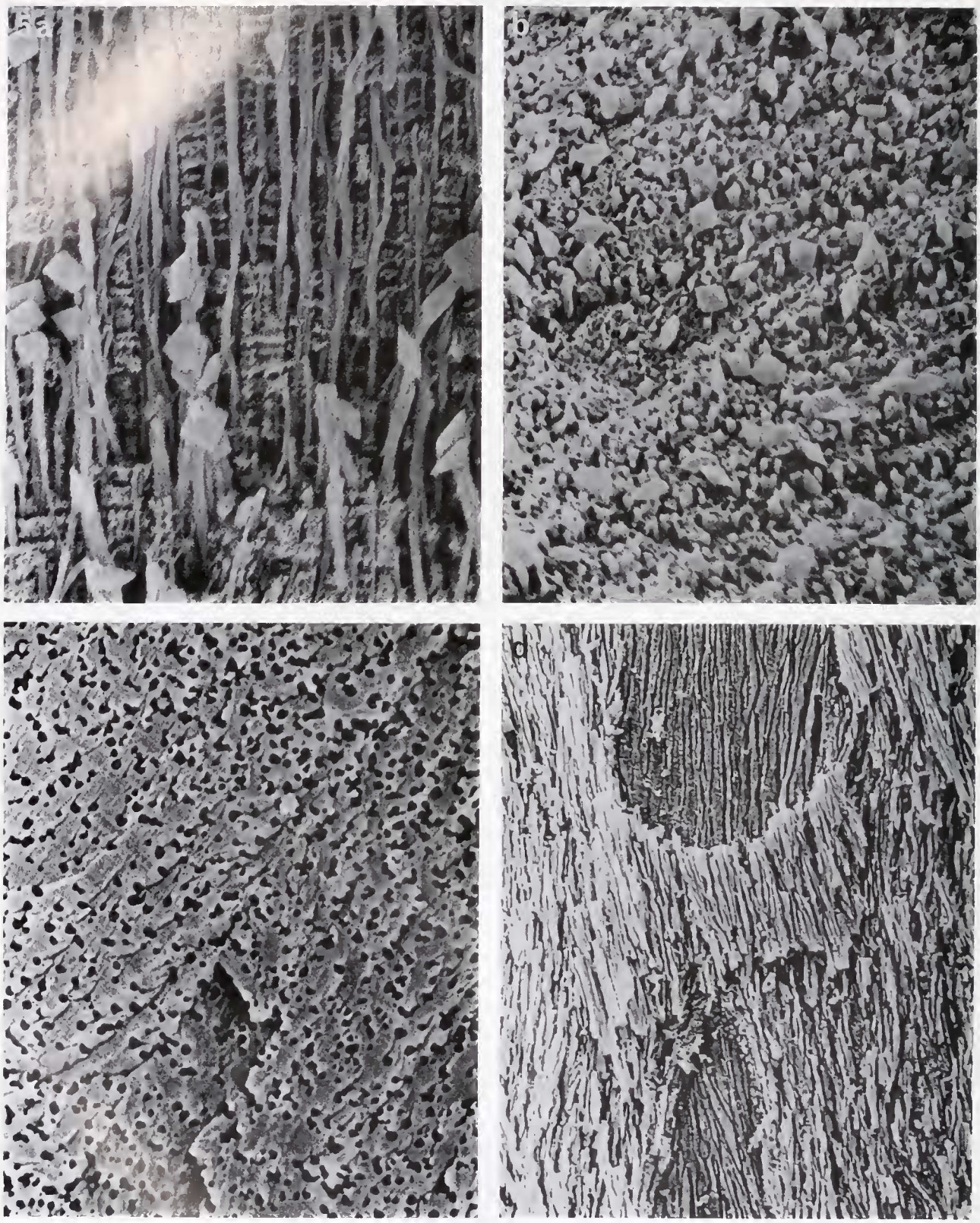

Figure 5. *Ellisella barbadensis.* (a) Ascorbic-acid etched, longitudinal fracture of an annulus with longitudinally oriented collagen fibers 0.2–0.4 μm diameter. Fibers have been freed from eroded crystal aggregates (horizontal bars). Bipyramidal shapes are crystal flakes. 4700×. (b) Ascorbic-acid etched surface of a transversely cleaved annulus. Short, protruding, slightly smoothed ends of collagen fibers protrude from the

Genus Lophogorgia

The axis of individuals of *Lophogorgia cardinalis* is moderately mineralized (about 10%/dry weight) by a carbonate with a high magnesium content (Jeyasuria and Lewis, 1987; Esford and Lewis, 1990). The X-ray diffraction pattern is that of an amorphous material, but it produces a faint calcite diffraction pattern. That this carbonate is probably a high magnesium calcite is supported by atomic absorption spectrometric determinations of mole percentages of magnesium in the calcium/magnesium carbonate. Mineral deposits occur as plate loculi that are crescentic, multilaminar, branched plates of irregular outline. Simplistically, the laminae of the plates are formed by alternating high and low density (fibers per unit area of gorgonin) layers of longitudinally oriented collagen fibers. The dense collagen fiber layers are lightly mineralized; the sparsely fibered layers, heavily mineralized. The mineral, in the form of tiny mineral bodies (crystals?), occurs in the matrix between and around collagen fibers. Collagen fibers in the low density laminae are usually heavily calcified, to the point they are individually sheathed by mineral, and often several adjacent collagen fibers may be incorporated into one, usually plate-like mass. Extensive mineralization of these laminae effectively produces very thin mineral sheets. Densely fibered laminae contain tiny mineral bodies (crystals) in the matrix. Ledger and Franc (1987) found that, in the axial skeleton of the sea pen *Veretillum cynomorium*, calcite deposits initially nucleate extracellularly between collagen fibers. They grow, push aside the collagen fibers, and eventually surround and encapsulate them. Significantly, mineral does not form within the collagen fibril, and collagen is not involved in the nucleation process. The same process may be occurring here. That the high magnesium calcite deposits in *Leptogorgia virgilata*, *Lophogorgia cardinalis*, and *Gorgonia ventalina* are amorphous is not surprising, if all of the mineral is in a form similar to that noted in *Lophogorgia cardinalis*. Crystals surrounding the collagen fibers are very small at about 0.1 μm thick and a few microns long. They are not arranged in a sufficiently regular pattern to produce a strong calcite diffraction pattern, but a faint diffraction pattern typical of calcite is barely detectable on the negatives. Randomly oriented proteoglycan molecules within a highly hydrated gel matrix between cell layers may cause the precipitation of crystals in an amorphous form (Kingsley and Watabe, 1984). Another possible explanation for the amorphous nature of calcareous matrix of *Lophogorgia cardinalis* is that the collagen fibers have nucleation sites on their surface. In Goldberg's (1976) analysis of the chemistry of gorgonian axial skeletons, the presence of large amounts of glycosaminoglycans (GAGs) was observed in a heavily mineralized gorgonian. The GAGs may coat the individual collagen fibers and thus initiate nucleation of the fine granular crystals (Addadi *et al.*, 1987). This method of crystal deposition and nucleation on individual collagen fibers could account for the fine, granular, amorphous nature of the mineral in *Lophogorgia cardinalis*.

Lateral to the calcified laminae are relatively thick layers of gorgonin containing a high density of collagen fibers. The structure of the laminated plate loculi is reminiscent of the laminated safety glass of automobile windshields.

Lophogorgia cardinalis, like *Ellisella barbadensis*, occurs below wave base where it is subject to current only. These small (circa 15 cm), relatively highly branched, planar, almost fan-like organisms require considerable stiffness to resist the current and maintain the polyps in feeding position in the water column. Their axial skeletons are among the stiffest found in Caribbean gorgonians (Esford and Lewis, 1990), and their torsion moduli are also high (Jeyasuria and Lewis, 1987). Laminated structures are often stiffer than comparable uniform composites, but it is the interconnectedness of the shield loculi that influences the stiffness. The extent and thickness of interconnections between individual plates probably also modulates resistance to twisting forces. Individual, separated, plate loculi embedded in gorgonin would not impart much resistance to compression. Interconnections between large numbers of them, effectively forming a mineral reticulum, would probably be quite resistant to compression. Such a reticulum would also produce considerable resistance to torsional forces.

SEMS of the axis of individuals of *Lophogorgia cardinalis* exhibit considerable artifactual disruption. Splits, cracks, and gaping spaces are common. Almost any treatment aimed at revealing one of the mineral or organic components of the axis will cause major structural perturbation. Removing the mineral phase with acids causes collagen to swell. This swelling disrupts structural integrity particularly along planes of weakness. Since the organic

eroded carbonate crystal surface. Carbonate forms irregular ring patterns around some of the collagen fibers. Bipyramidal shapes are crystal flakes. 4700×. (c) Bleach-etched surface of a transversely cleaved annulus. Collagen has been removed and reveals perforations in the carbonate crystal plates through which the fibers extend. Note the high density of the perforations and the fine diagonal striae with a period of about 0.2 μm that probably represent episodic carbonate deposition. 4700×. (d) Bleach-etched, longitudinally fractured outer surface layers of an annulus. The multilaminar layer of fine-grained, longitudinal crystals caps the annular crystal aggregates and forms nucleation sites for subsequent annulus formation. 1900×.

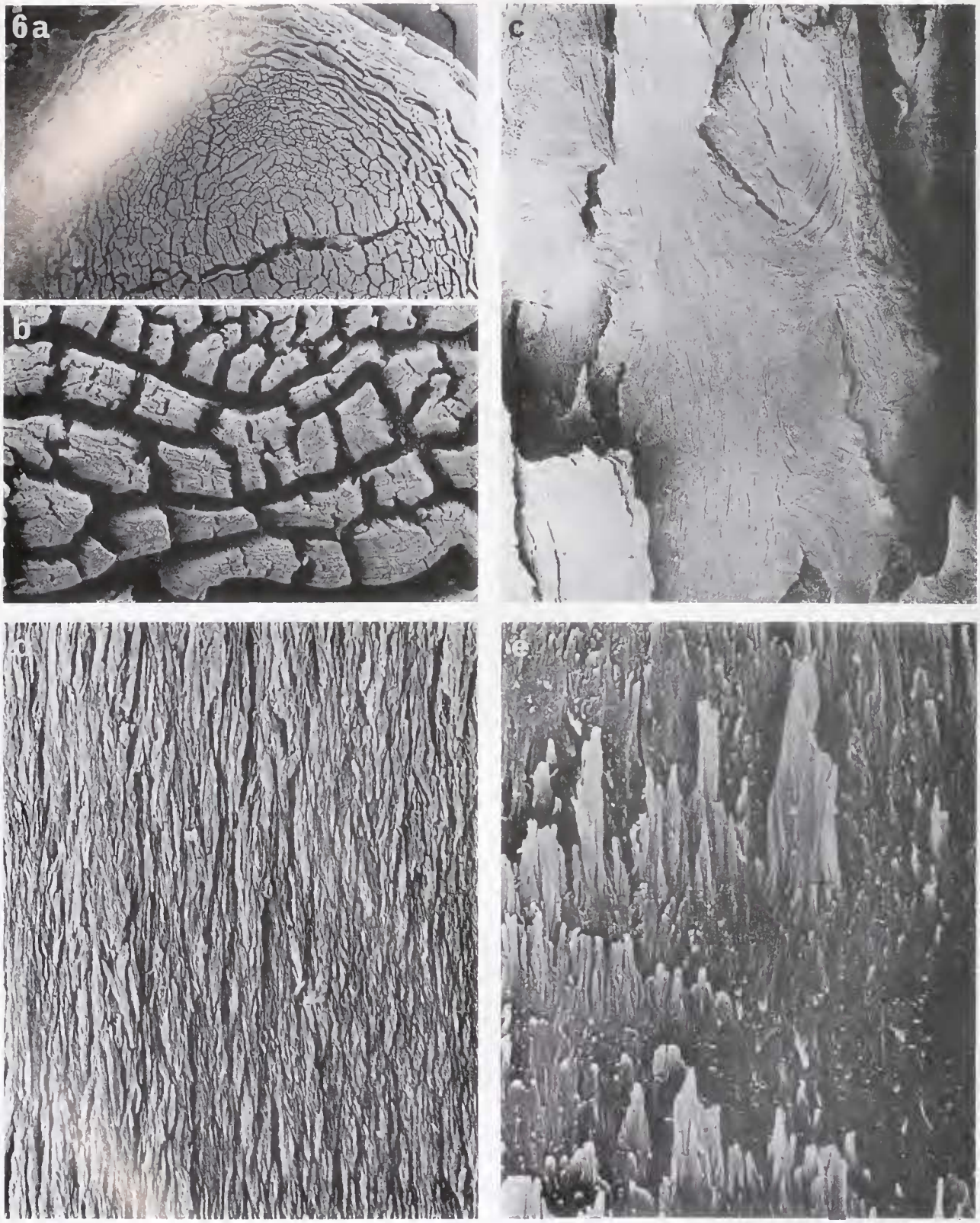

Figure 6. (a) Bleach-etched (gorgonin removed) cross section of the axis of *Lophogorgia cardinalis*. Numerous, generally crescentic calcareous loculi form a major component of the axis. 33×. (b) Bleach-etched cross-sectioned, crescentic loculi with artifactual fractures caused by removal of gorgonin support, rehydration and dehydration. 470×. (c) Bleach-etched, longitudinally fractured axis of *L. cardinalis* showing the outer surface of a plate loculus. Note its irregular outline and connections with neighbouring locular plates. 160×. (d) Bleach-etched, outer surface of a plate loculus with fine-grained, longitudinally oriented crystals. 2400×. (e) Interlocular gorgonin with layered, longitudinally oriented collagen fibers. 2400×.

phase is so large, about 90% (Esford and Lewis, 1990), its removal with hypochlorite also eliminates support for the mineral phase. Since the mineral is in the form of delicate plates and single, free-standing, carbonate-sheathed collagen fibers, this undermining has rather drastic effects. These are further exacerbated by the swelling caused by hypochlorite and the subsequent dehydration required for examination by SEM. Fortunately, the deleterious effects of these treatments do not appear to be uniformly distributed. They appear to be limited to the large cheeks, cracks and gaps, and large areas of axis remain in what appears to be the "original" condition, with one phase or another removed.

All species

Polarized microscopy of thin longitudinal sections indicates that the 12 species examined have a fibrillar component oriented in parallel with the longitudinal axis of the colony only.

Calcareous material could also be extracted from the axes of all species examined, though in some species the minute amounts of carbonate extracted were probably from the hollow core of the axis and are possibly nonbiogenic in origin. X-ray diffraction indicates that the major form of carbonate occurring in these gorgonian axes is calcite. Positive Aliziran red staining, in combination with calcium and magnesium proportions derived from atomic absorption spectroscopy (Jeyasuria and Lewis, 1987; Esford and Lewis, 1990), indicate that the carbonate is in the form of high magnesium calcite. This also applies to those crystal forms that produced an amorphous X-ray diffraction pattern.

Ellisella barbadensis and the three species of *Plexaurella* have large, regular crystalline, high magnesium calcite aggregations in their axes. In these species, visible mineralization was highest in the cross sections of tips and lowest in bases. This correlates well with the findings of Esford and Lewis (1990) that the Young's modulus or stiffness of axial skeletons was higher in the tips than in the bases of *Ellisella barbadensis* and the species of *Plexaurella* that they examined.

Axes of *Lophogorgia cardinalis* and *Leptogorgia virgilata* contain thin carbonate plates that produce amorphous X-ray diffraction patterns. Mole percentages of magnesium in the carbonate (Jeyasuria and Lewis, 1987) are indicative of high magnesium calcite. Atomic absorption spectroscopy also indicates high magnesium calcite as the mineral form for the amorphously diffracting small crystalline aggregates from *Gorgonia ventalina*.

Species of *Pseudopterogorgia* have calcitic fibroids between or around collagen bundles. Fibroid mineralization probably occurs between collagen bundles which act as templates. This results in the fibrous appearance of the mineral.

In the preceding genera, mineralization is biologically controlled. The organic matrix acts to constrain the exterior surface of the mineralized aggregate, making a finite variety of shapes possible. This biogenic process is characterized by a mineralization site sealed off from the environment by a barrier through which ions cannot freely diffuse (Lowenstam and Weiner, 1989). Space delineation is a fundamental part of the cellular machinery that controls mineralization (Wilbur, 1984). Biological control of the type and form of mineral deposited is undoubtedly important for structural reasons.

In the remaining species, mineralization is thought to be nonbiogenic and occurs in an open environment such as the hollow central region of the axis. The physical characteristics of nonbiogenic mineralization include a morphological structure analogous to their organic counterparts, as well as randomly clumped aggregates of varying sizes (Lowenstam and Weiner, 1989). These diagnostic features are readily observed in the scanning electron micrographs of *Eunicea tourneforti, Muricea muricata,* and *Plexaura flexuosa*. Nonbiogenic mineralization may be induced by a relatively minor perturbation, such as the introduction of biologically produced metabolic endproducts, the release of particular cations by the cell, or even by the construction of a charged surface such as a cell wall (Wainwright, 1988). Basically, no specialized cellular or macromolecular mechanism regulates this precipitation.

A characteristic of nonbiogenic mineralization is that the type of mineral formed is a function of the environmental conditions in which the organism occurs, as much as of the biological processes involved in its formation (Wainwright, 1988). Thus, the same organism in a different environment can form different minerals (Lowenstam and Weiner, 1989). A mineral shift, whereby a species may precipitate different mineral forms in response to external stimuli, was demonstrated by Lowenstam (1964), who found that temperature and salinity were the dependent variables in the ratios of aragonite to calcite in certain (*Octocorallia*) species of *Holaxonia*. He concluded that, at mean seawater salinity, the aragonite/calcite ratio of the carbonate skeleton increased with the ambient temperature.

In this study, species exhibiting what we regard as nonbiogenic mineralization that probably occurs only in the hollow core of the axis, contain minute quantities of carbonate and less than ½ of 1% total Ca + Mg/dry wt of axis (Jeyasuria and Lewis, 1987; Esford and Lewis, 1990). X-ray diffraction patterns from minute quantities of mineral reveal calcite in *Muricea muricata*, aragonite in *Eunicea tourneforti*, and amorphous in *Plexaura flexuosa*. Here, "amorphous" means only that no recognizable diffraction pattern is generated. Lowenstam (1964) found that carbonate extracted from *Plexaura flexuosa* is

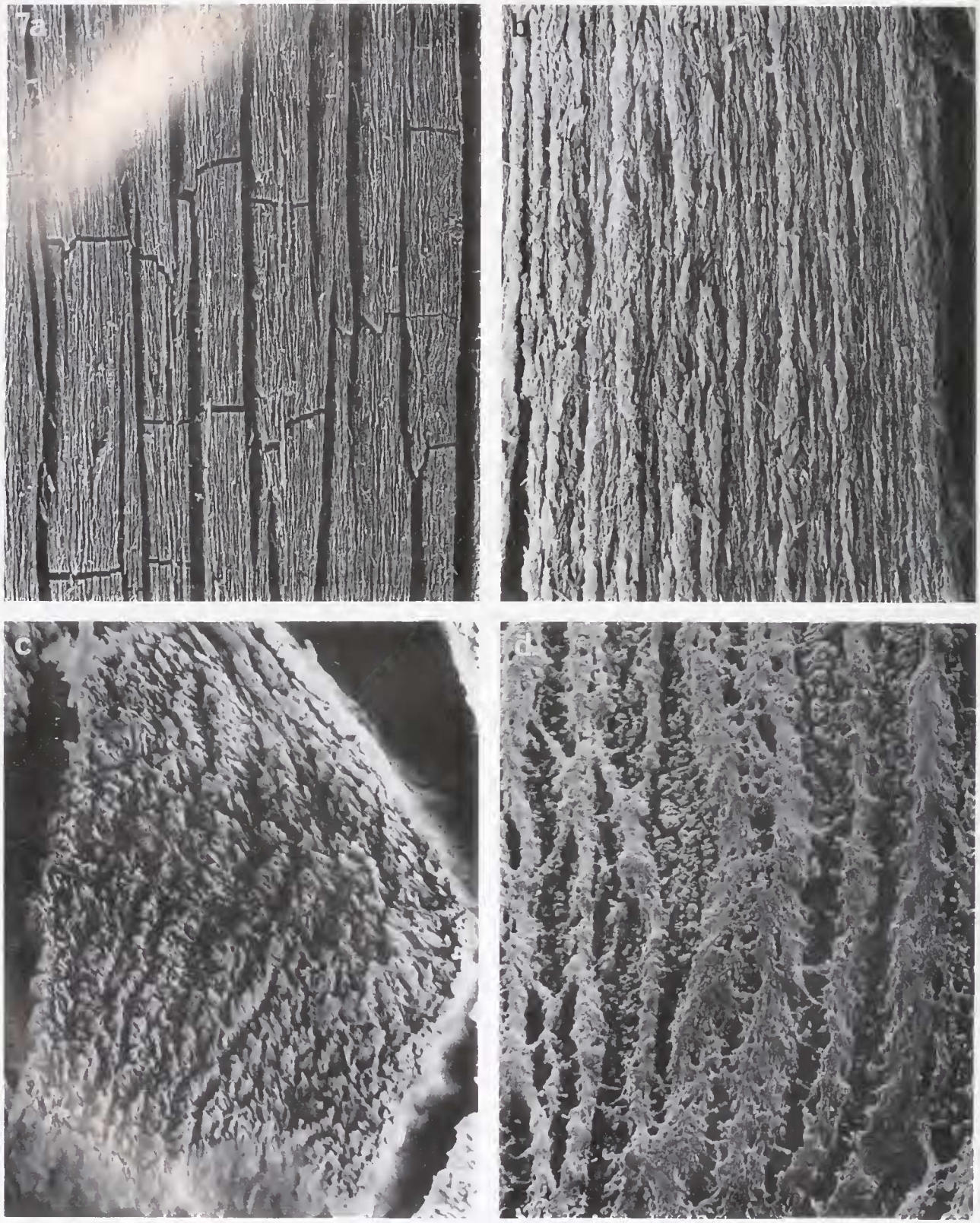

Figure 7. (a) Bleach-etched edges of longitudinally cleaved plate loculi show the laminar organization of mineral and gorgonin layers of *Lophogorgia cardinalis*. Note the lateral branching and fusion of calcified connections with adjacent plates. 470×. (b) Bleach-etched edge of a longitudinally cleaved plate loculus reveals rope-like rows of fine-grained, longitudinally oriented fine crystaline bodies. Grooves represent areas

Table II

A comparison of mechanical properties, associated structures, and habitat in Ellisella barbadensis, Lophogorgia cardinalis, Plexaurella, *and non-mineralized specimens*

Species	Tm	Ym	% Ca/Mg	Structure	Habitat
E. barbadensis	8.6	90	30	fiber-reinforced concentric rings	current
L. cardinalis	5.8	40	12	laminae of sheathed collagen fibers	current
Plexaurella	3.5	35	20	fusiform loculi separate from collagen	surge
Others	0.7	5–10	>1	no mineral	surge

Tm: torsion modulus, a measure of resistance to twist in Gdynes/cm^2.
Ym: approximated Young's modulus, a measure of stiffness.
% Ca/Mg: percentage of carbonate/dry weight of axis (Jeyasuria and Lewis, 1987; Esford and Lewis, 1990).

aragonitic, but obtained no definitive stain reaction. He also makes reference to an aragonitic axis in *Plexaurella dichotoma*, which is at odds with our determinations of high magnesium calcite in three other species from that genus.

A preliminary comparison of structure and some associated mechanical properties is instructive (Table II). The following tentative conclusions can be drawn from the table: (1) Unmineralized gorgonin has low stiffness and low torsion resistance. (The "other species" are exemplary.) (2) Mineral is used in different ways to stiffen gorgonin (compare the "Structure" with "Ym" columns). (3) Mineral content alone does not determine stiffness. (*Lophogorgia cardinalis* with half the mineral content of *Plexaurella* is somewhat stiffer.) (4) Mineral content alone does not determine torsional resistance. (*Lophogorgia cardinalis*, with half the mineral content of *Plexaurella*, is much more resistant to twist). (5) Incorporation of collagen into the mineral phase increases stiffness. (Individuals of both *Ellisella barbadensis* and *Lophogorgia cardinalis*, are stiffer than the *Plexaurella*, despite *Lophogorgia cardinalis* having far less mineral). (6) Separation of the mineral phase from the collagen fibers lowers both Young's and torsion moduli. Species of (*Plexaurella* have lower moduli than *Ellisella barbadensis* and *Lophogorgia cardinalis*). (7) Higher torsional, tensional, and compressional stiffness is used in current than in surge. (Consistently higher Ym and Tm are found in the two species that occur in the current zone). (8) The reinforced concrete style of mineralization found in *Ellisella barbadensis* produces very high stiffness and very high resistance to torsion. (9) The interconnected, lamellar, shield-like plates of sheathed collagen fibers produces moderately high stiffness and moderately high resistance to torsion with relatively little mineral in *Lophogorgia cardinalis*. (10) The lenticular crystalline loculi of species of *Plexaurella* are separate from the collagen fibers and produce moderately high stiffness, but moderately low resistance to torsional forces.

Acknowledgments

We thank L. Stobbs and J. Van Schagen of the Vineland Agriculture Canada Research Station for assistance with SEM, U. Brand, Department of Geological Sciences and M. Richardson, Department of Chemistry, Brock University, for assistance with X-ray diffraction and the Prime Minister's Office of the Government of Trinidad-Tobago for permission to collect gorgonians for this research.

Literature Cited

Addadi, L., J. Moradian, E. Shay, N. G. Maroudas, and S. Weiner. 1987. A chemical model for the cooperation of sulphates and carboxylates in calcite crystal nucleation: relevance to biomineralization. *Proc. Nat. Acad. Sci. USA* **84**: 2732–2736.

Barnes, R. D. 1980. *Invertebrate zoology, 4th Edition.* Pp. 164–169. Saunders College/Holt Rhinehart and Winston, Philadelphia.

Barnowski, T. F. 1991. SEM of the heavily calcified axial skeletons of three genera of Caribbean gorgonians. *Hons. B.Sc. Thesis.* Brock University, St. Catharines, Canada. 137 pp.

Bayer, F. M. 1961. *The Shallow Water Octocorallia of the West Indian Region.* Marinus Nijhoff, The Hague. 373 pp.

Carter, J. G., and G. R. Clark II. 1985. Classification and phylogenetic significance of molluscan shell microstructure; in *Mollusks: Notes for a short course*, D. J. Bottjer, C. S. Hickman, and P. D. Ward, eds. University of Tennessee, Dept. of Geological Sciences, Studies in Geology 13. 305 pp.

Chalker, B. E., and D. L. Taylor. 1978. Rhythmic variations in calcification and photosynthesis associated with the coral *Acropora cervicornis*. *Proc. R. Soc. Lond. B. Biol. Sci.* **201**: 179–189.

Esford, L. E., and J. C. Lewis. 1990. Stiffness of Caribbean gorgonians (Coelenterata, Octocorallia) and Ca/Mg content of their axes. *Mar. Ecol. Prog. Ser.* **67**: 189–200.

Gladfelter, E. H. 1984. A comparison of monthly rates of linear extension and calcium carbonate accretion measured over a year. *Coral Reefs* **3**: 51–57.

from which collagen has been removed. X-ray diffraction pattern is amorphous. 1400×. (c) Bleach-etched, transversely fractured plate loculus of *Lophogorgia cardinalis*. Note rows of carbonate, sleeve-like structures, many with hollow centres. Dark areas between rows remain from gorgonin removal. Carbonate is probably deposited around individual collagen fibers. (d) Acid-etched, transversely fractured plate loculus. Fused and melted ends of dense collagen-gorgonin layers form elevated ridges. Valleys contain ends of acid-eroded calcareous tubes. 2400×.

Goldberg, W. M. 1973. Evidence of sclerotized collagen from the skeleton of a gorgonian. *Comp. Biochem. Physiol.* **49:** 525–532.

Goldberg, W. M. 1976. Comparative Study of the chemistry and structure of [illegible] and antipatharian coral skeletons. *Mar. Biol.* **35:** 253–[illegible]

Jeyasuria, P., and J. C. Lewis. 1987. Mechanical properties of the axial skeleton in gorgonians. *Coral Reefs* **5:** 213–219.

Kingsley, R. J., and N. Watabe. 1984. Calcium uptake in the gorgonian *Leptogorgia virgulata*. The effects of ATPase inhibitors. *Comp. Biochem. Physiol.* **79A:** 487–491.

Kingsley, R. J., and N. Watabe. 1987. Role of carbonic anhydrase in calcification in the gorgonian *Leptogorgia virgulata*. *J. Exp. Zool.* **241:** 171–180.

Koehl, M. A. R. 1982. Mechanical design in spicule reinforced connective tissues: Stiffness. *J. Exp. Biol.* **98:** 239–267.

Ledger, P. W., and S. Franc. 1978. Calcification of the collagenous axial skeleton of *Vertillum cynomorium* Pall (Cnidaria: Pennatulacea). *Cell. Tissue Res.* **192:** 249–266.

Leversee, G. J. 1969. Composition and function of the axial skeleton in the gorgonian coral *Leptogorgia virgulata*. *Am. Zool.* **9:** 1115 (abstract).

Lowenstam, H. A. 1964. Coexisting calcites and aragonites from skeletal carbonates of marine organisms and their strontium and magnesium contents. Pp. 373–404 in *Recent Researches in the Fields of Hydrosphere, Atmosphere and Nuclear Geochemistry*, Y. Miyake, and T. Koyama, eds. Maruzen Co., Ltd., Tokyo.

Lowenstam, H. A., and S. Weiner. 1989. *On Biomineralization*. Oxford University Press. New York. 324 pp.

Muss, M. L. 1964. The phylogeny of mineralized tissue. *Int. Rev. Gen. Exp. Zool.* **1:** 297–331.

Muzik, K., and S. A. Wainwright. 1977. Morphology and habitat of five fijian fans. *Bull. Mar. Sci.* **27:** 308–337.

Pearse, V. B., and L. Muscatine. 1971. Roles of symbiotic algae (zooxanthellae) in coral calcification. *Biol. Bull.* **141:** 350–363.

Ramsaroop, D. 1977. Common shallow-water gorgonians of Trinidad. *Living World* **2:** 21–32.

Roberts, H. H., S. N. Murray, and J. N. Suhayda. 1975. Physical processes in a fringing reef system. *J. Mar. Res.* **33:** 233–260.

Simkiss, K., and K. M. Wilbur. 1989. *Biomineralization: Cell biology and mineral deposition*. Academic Press, Inc. Toronto. 337 pp.

Telesnicki, G. J. 1990. A comparative study of the structure of gorgonian axial skeletons. *Hons. B.Sc. Thesis*. Brock University, St. Catharines, Canada. 140 pp.

Vincent, J. F. V. 1981. *Structural Biomaterials*. MacMillan Press Ltd., London. 206 pp.

Wainwright, S. A., W. D. Biggs, J. D. Currey, and J. M. Gosline. 1982. *Mechanical design in organisms*. Princeton University Press, New Jersey. 423 pp.

Wainwright, S. A. 1988. *Axis and Circumference: the Cylindrical Shape of Plants and Animals*. Harvard Univ. Press., Cambridge. 132 pp.

Weiner, S., W. Traub, and H. A. Lowenstam. 1983. *Biomineralization and Biological Metal Accumulation*, P. Westbroek and E. W. de Jong, eds. Dordrecht, Netherlands. Pp. 205–224.

Weise, L. 1988. *Cell and Tissue Biology: A Textbook of Histology, Sixth Edition*. Urban and Schwarzenberg, Inc., Baltimore, MD. U.S.A. 1158 pp.

Wilbur, M. K. 1984. Many minerals, several phyla, few considerations. *Am. Zool.* **24:** 839–845.